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The Electroretinogram of *Heliconius erato* (Lepidoptera) and Its Possible Relation to Established Behavior Patterns[1,2]

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(Plates I & II; Text-figures 1-6)

[This paper is one of a series emanating from the William Beebe Tropical Research Station of the New York Zoological Society, at Simla, Arima Valley, Trinidad, West Indies. The station was founded in 1950 by the Zoological Society's Department of Tropical Research, under Dr. Beebe's direction. It comprises of 200 acres in the middle of the Northern Range, which includes large stretches of government forest reserves. The altitude of the research area is 500 to 1,800 feet, and the annual rainfall is more than 100 inches.

[For further ecological details of meteorology and biotic zones, see "Introduction to the Ecology of the Arima Valley, Trinidad, B.W.I.," by William Beebe, Zoologica, 1952, 37 (13) 157-184.

[The success of the present study is in large measure due to the cooperation of the staff at Simla, especially of Jocelyn Crane, Director, who contributed much of her knowledge of the organisms studied and helped with the recording of observations].

INTRODUCTION

NUMEROUS orders of insects such as the Odonata (*e.g.*, Mazokin-Porshniakov, 1959) Orthoptera (*e.g.*, Walther, 1958; Jahn & Wulff, 1942), Coleoptera *(e.g.,* Jahn & Verner, 1941), Diptera *(e.g.,* Autrum *et. al.,* 1961) and Hymenoptera (*e.g.,* Goldsmith, 1960), have been examined to determine the character of the insect electroretinogram. With the exception of Jahn & Crescitelli's (1939) early work on the *Cecropia* moth, little, if any, electrophysiological data concerning the visual processes in the Lepidoptera have been obtained. This is particularly surprising in view of the fact

that their bright coloration, proved visual orientation (*e.g.*, Crane, 1955; Ilse & Vaidya, 1956; Magnus, 1956) and rapid flight patterns all attest to an acute visual system and well developed color discrimination. With these facts in mind, and as part of a continuing investigation into the biology of the heliconiine butterflies, this study into the character of the electroretinogram of *Heliconius erato hydara* Hewitson (1869) (see Kaye, 1921) was conducted.

This species of butterfly common in the neotropics has already received careful attention from several viewpoints. Crane (1954) has described the spectral reflection characteristics of its wing coloration in detail. Briefly, it is a small (2½″ wing span), black butterfly, with brilliant scarlet patches on both the upper and lower surfaces of the forewings. Its phylogenetic position is being established by Emsley as part of this over all study. Genetical studies of the described Trinidad form, and the highly polymorphic forms found widely distributed over the South American continent, are being studied by Emsley & Sheppard (*e.g.*, Beebe, 1955). Crane (1955, 1957) has made a notable contribution in her studies of the behavior patterns and the role that visual orientation plays in the release of these patterns in this butterfly. These studies conclusively demonstrated its preference for the color orange as opposed to various shades of gray, and other hues, in their feeding behavior. On the other hand, red appears to play a major role in species identification and serves as a key element in the release of mating behavior. Other investigators, notably Eltringham (1933) and Ilse & Vaidya (1956), working with other species, have demonstrated visual orientation in butter-

fly feeding behavior. Magnus (1956) demonstrated that in certain butterflies, a cylinder with vertical colored and black bands, if rotated, would attract male butterflies. Increasing the speed of rotation increased the attractiveness of the cylinder.

Several authors (*e.g.,* Jahn, 1946; Crescitelli & Jahn, 1939) have discounted the existence of any color component within the electroretinogram of insects. Recently Autrum (1960) has contested this viewpoint.

Materials and Methods

Butterflies used in these experiments were captured in the wild and maintained in large outdoor insectaries. This technique provided a ready source of healthy material. (See Crane & Fleming, 1953, for description of these cages).

Since *Heliconius erato* settles quickly into a life within the insectaries, it was practicable to observe its behavior patterns and where necessary to record with high speed photography its wing beat frequency, etc.

Electroretinograms were recorded using steel electrodes (tip diameter *ca.* 15 μ. Potentials were amplified with a Grass P-6 D.C. amplifier, presented on a Tektronix dual beam oscilloscope, and recorded with Grass C-4 camera.

The stimulus light source was a 100 watt, GE T8½/9 incandescent light (operated through a constant voltage transformer) with a color temperature of 2960°K. The light was focused into the ocular of a compound microscope, mounted horizontally inside a darkened and electrostatically shielded enclosure. A 16 mm. objective lens focused a spot (*ca.* .5 mm. diameter) of light upon the cornea of the specimen.

Control of duration of stimulus was provided by a Compur type shutter and a rotating notched disk which could provide a flickering light source. Intensity was varied by means of a series of Bausch and Lomb neutral density filters. Monochromatic stimulation was accomplished by means of twelve "Balzers Liechtenstein" narrowband interference filters covering the 420 mμ to 695 mμ portion of the spectrum.

Energy levels for each wavelength were computed on the basis of spectral emission curves provided by the lamp manufacturers, and the transmission curves of the interference filters (provided by Photovolt Corp.). Appropriate neutral density filters were then combined with each interference filter so as to obtain a nearly equal energy level at each wavelength.

Absolute light intensity values were obtained using rather crude "grease-spot" photometric techniques and hence should be considered only approximate.

In all recordings an upward beam deflection indicates a negative polarity of the active elecrode.

The electrophysiology laboratory was maintained at a constant temperature of 68° F.

Results

The electroretinogram (ERG) of *Heliconius erato* quickly showed itself to be of a highly variable nature. It soon became apparent, however, that this variation could be fitted to a diurnal pattern. ERGs produced during the night hours, early morning and occasionally during the late afternoon, were found to be of one type. Another type of ERG was produced from mid-morning to some indefinite time late in the afternoon or evening. Plate I, fig. 1, shows selected results from an experiment which consisted of withdrawing a sample individual from the population within the insectary, every hour from 6 A.M. to midnight. It was found practicable to remove an individual from its "normal" environmental conditions and record the first ERG in only six to ten minutes. It will be noted that the individuals sampled at 0600, 0800, and 2400 presented an ERG quite different in waveform from that recorded at 0900 and the remainder of the day. It is worth noting that the individual whose response is shown in 0800 altered the character of his response during the ensuing hour while mounted in the experimental set-up and by 0845 demonstrated a response quite like that illustrated in the figure for the organism sampled at 0900. The "night" response (*e.g.,* 0600 wave form) can be characterized as follows:

The initial response consists of a sharp rise in negativity, followed quickly by a very slight drop. This is followed by a sustained negativity which, upon intense stimulation, may show a slight, continual increase in magnitude (for stimulus durations up to 1 second). Upon discontinuing the stimulation there may be a small irregular-appearing increase in negativity (or "off" response).

The "day" response (*i.e.,* 0900) can be described as often having a brief positive initial response ("A" wave), particularly upon stimulation with long wavelengths, followed immediately by the initial negative component or "B" wave which possesses a somewhat less steeply rising slope, and often contains a slight irregularity. Following this depolarization there is a rapid repolarization which returns the recorded potential nearly to or below (positive) the resting potential. Then follows a sustained response or "C" wave which describes a gradually rising curve with a constantly decreasing slope. When stimulation is discontinued, a sudden increase in negativity, or "D" wave, is observed.

In the course of these experiments well over a hundred individuals of *H. erato* have been subjected to ERG experimentation. All wave forms obtained in the course of these experiments can be described as either "day," "night" or transitional. Plate I, fig. 2, illustrates a portion of a natural transition from "night" to "day." In this instance the butterfly was removed from the insectary and "normal" environmental conditions just prior to 0800 and mounted in the darkened, temperature-controlled environment of the laboratory. The only light to which it was exposed was the actual test stimuli. Other experiments utilizing a comparable technique, when conducted at other times of the day, failed to reveal any comparable change in wave form. This transition cannot, therefore, be attributed to dark adaptation, temperature effects, etc.

It is extremely difficult to maintain these organisms for prolonged periods of time under artificial environmental conditions. I was successful, however, in keeping three individuals alive for nearly three days in constant temperature (84° F.) and constant shadowless illumination (220 foot candles). At the end of this period the butterflies were sampled at 0800, 0830 and 1000 and demonstrated the "normal" transformation associated with this time of day (Plate I, fig. 3). On the basis of this experiment it is suggested that this transformation is regulated by a biological clock. Histological studies demonstrated that, as in *Dytiscus* (Jahn & Verner, 1941), retinal pigment distribution is not involved in the transition. In general it was observed that this transformation from "night" to "day" occurred very nearly simultaneously in all butterflies tested. That is to say, transition was occasionally observed to commence as early as 0745, but more commonly did not begin until about 0815. The transition was nearly always complete by 0900, but occasionally not until 0930. The other shift was not nearly so regular. Occasionally individuals were found to have returned to the "night" response by 1300. More commonly, however, this transition did not take place until the very late evening and frequently was found to occur a considerable time after the butterfly had gone to sleep for the night.

Having thus established that the ERG consists of two superficially distinct wave forms; it became necessary to determine the characteristics of each type individually—the spectral response, flicker fusion frequency, variation in magnitude with respect to stimulus intensity, etc. The "day" response is discussed first.

Measurements of the magnitude of the initial negative or "B" wave component of the day response as a function of stimulus wave length produce the luminosity curve presented in Text-fig. 1. This curve shows a single peak at about 528 mμ, corresponding closely to the peak in spectral sensitivity obtained by Goldsmith (1960) for the worker honey bee. There is some indication that there may indeed be a second peak in the ultraviolet. However, since equipment was not available for providing stimulation in this portion of the spectrum, no conclusions are presently possible.

Analysis of the magnitude of the "D" wave or "off" response show that it normally bears a constant relationship to the magnitude of the "B" wave. Thus twelve "day" individuals were exposed to six different intensities of white light stimulation with relative strengths as follows: 1, 4, 16, 64, 256 and 1,024. The average value for the magnitude of the "off" reponse, expressed as a fraction of "B" wave magnitude, were as follows: 28%, 33.5%, 31%, 32.5%, 25% and 27%, respectively. This variation of $\pm$ 4.2% is well within the range of experimental variation for the type of equipment and number of individuals tested.

If, on the other hand, the magnitude of the "off" response as a percentage of "B" wave magnitude is computed for the response to various wave lengths of equal energy, monochromatic stimulation, a marked deviation from a constant relationship becomes readily apparent. Thus, based on eleven individuals, the average magnitude of the "off" response may vary from 19% of "B" wave magnitude up to a maximum of 46%. This represents a variation more than twice as great as the variation recorded in response to various white light stimulus intensities. Moreover, it will be noted that this variation expresses a fairly smooth, action spectrum, as indicated in Text-fig. 2, with a maximum at about 636 mμ. Obviously this action spectrum cannot be accounted for in terms of an intensity phenomenon, but rather indicates the responsiveness of the "D" wave to the activity of some component with a spectral sensitivity basically different from the receptor responsible for producing the "B" wave.

Various authors have maintained that the insect ERG does not show characteristics which are unique for the stimulus wave length. That is to say, all variations in the shape of the wave form can be attributed to an intensity phenomenon, and if proper stimulus intensities are chosen, so that the response to each wave length is of equal magnitude, then the wave forms become identical, regardless of the wave length of the stimulus. The triple recording in Plate I, fig. 4, demonstrates the response to stimulation with three widely separated wave lengths in which the
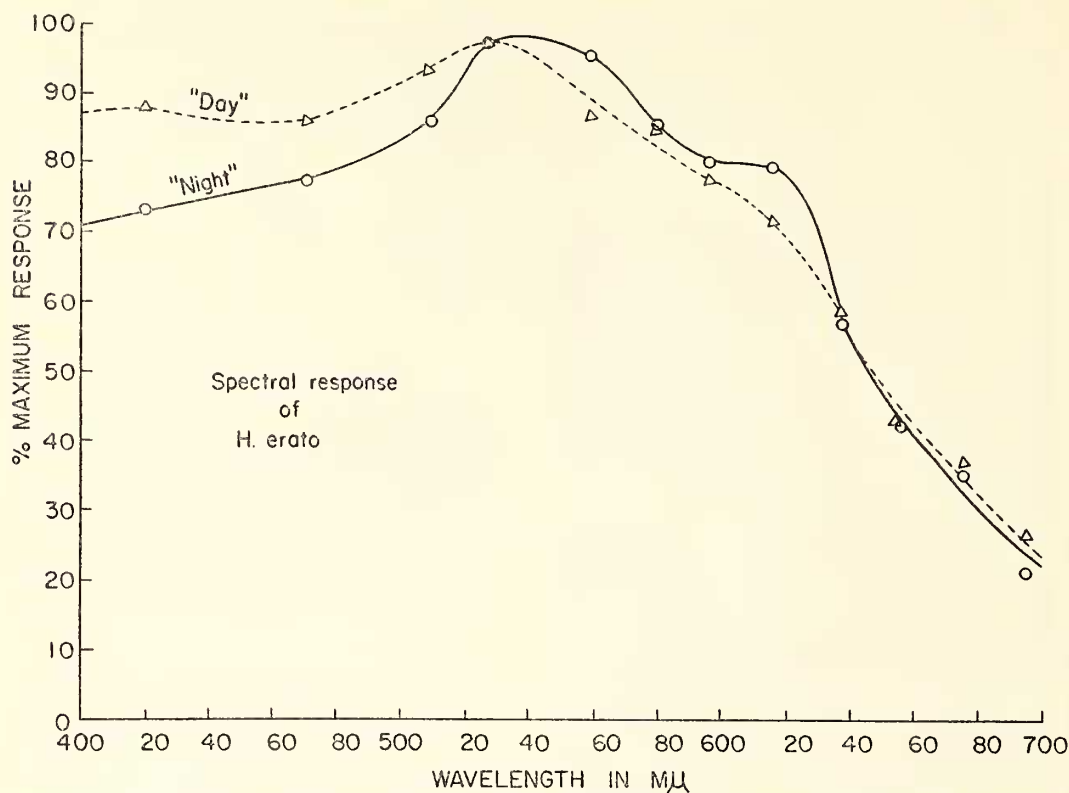
TEXT-FIG. 1. Spectral response (luminosity) curves, based upon the responses of 14 individuals in the "night" phase, and 11 individuals in the "day" phase. Magnitude of B wave used as criterion. Since the absolute magnitude of electrical potentials recorded can vary considerably among individuals, the magnitude of each individual's response to 12 wavelengths was measured as a percentage of the response to the wavelengths eliciting the greatest response. These percentages were then averaged and plotted.

intensities were adjusted so as to produce as nearly equal magnitude "B" waves as possible; it will be observed that each color presents a markedly different wave form. Also, it will be noted that this variation in wave form takes the form of a smooth series throughout the range of wave lengths tested and does not, in fact, show any tendency of the wave form to become identical on either side of the "B" wave maximum of 528 mμ. It is, therefore, concluded that the "day" ERG possesses a distinct and unique component within the character of the wave form which may be attributed to stimulus wavelength alone.

Other characteristics of general interest concerning the nature of the "day" visual response are presented in Text-figs. 3 & 4 and Plate II, fig. 5. Text-fig. 3 presents a dark adaptation curve based on the responses of three individuals. The technique involved in obtaining this curve was to focus a very intense white light upon the head of the butterfly for at least ten minutes. The light was then extinguished and the electrophysiological threshold (*i.e.*, minimum stimulus intensity required to produce any electrical response) was

determined after various time intervals in total darkness. This curve demonstrates the fairly rapid dark adaptation that takes place in *H. erato.*

Text-fig. 4, is a typical flicker fusion versus stimulus intensity plot. As is usual, flicker fusion frequency is related to light intensity. A maximum F.F.F. of 165 cycles/sec. has been recorded, and characterizes the butterfly eye as moderately fast, certainly not the equal of the 250 cps Autrum (1958) recorded from the fly *Calliphora,* but, on the other hand, much faster than the 20 cps he reports for the grasshopper *Tachycines.* The response to a flickering light, however, shows a sustained negativity with the flicker responses superimposed. This type of response Autrum has characterized as typical of the "slow" eye. One other interesting phenomenon is the fact that the response to a flickering light shows two critical frequencies (Plate II, fig. 5).

The usual critical frequency or F.F.F. represents the point at which the eye begins to respond discretely to each individual flash. These re-
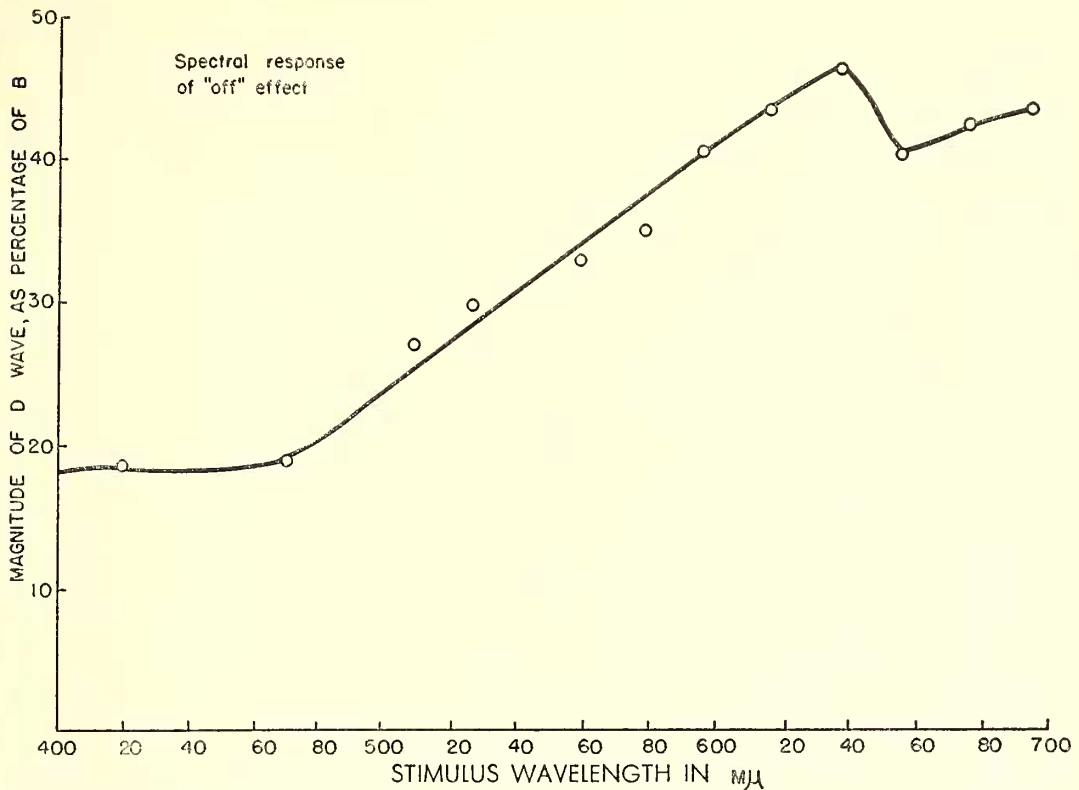
TEXT-FIG. 2. D wave magnitude as a function of stimulus wavelength. Each D wave was measured as a percentage of the B wave magnitude elicited by the same 100 msec. test flash. The curve represents values obtained by averaging the responses of 11 "day" individuals.

sponses can be shown to be "on" responses. At some other much lower frequency (20-26 cps) it can be demonstrated that another significant phenomenon takes place. This is in essence a transition from "on" to "off" responses.

As for the night response, Text-fig. 1 presents a luminosity curve derived from the magnitude of the initial negative wave. It is apparent that this action spectrum has two maxima. One is identical with that of the "day" response (528 mμ) but another new maximum develops at 616 mμ with a plateau connecting these two peaks. It is worth noting that this new peak in the red corresponds approximately to the peak in the luminosity curve derived from the "D" wave of the "day" response (Text-fig. 2). No satisfactory "D" wave luminosity curve can be computed for the "night" response because of the small and irregular nature of this component. It is, however, worth noting that the maximum F.F.F. observed in an eye producing the night response was 90 cycles per second and that no second critical frequency is observed.

The response characteristic of night, quite un-

like the "day" response, does not have a color component apparent in the wave form. Plate II, fig. 6, shows the response to the same three wave lengths as utilized in Plate I, fig. 4. It will be noted that it is possible to cause the wave form obtained from any wavelength within the visual spectrum to coincide with the wave form produced by any other wavelength.

Text-fig. 5 contrasts the increase in response magnitude accompanying an increase in stimulus magnitude in both "day" and "night" responses. A given increase in stimulus intensity produces a greater increase in electrical response in the "night" eye than in the "day" eye. No statistically significant difference between dark - adapted thresholds of "day" and "night" eyes could be detected.

In conjunction with these electrophysiological experiments, certain observations upon normal behavior patterns were conducted. Text-fig. 6 demonstrates graphically the results of these observations made upon the frequency of certain types of activity throughout one day. Environmental conditions (*i.e.*, light and temperature)
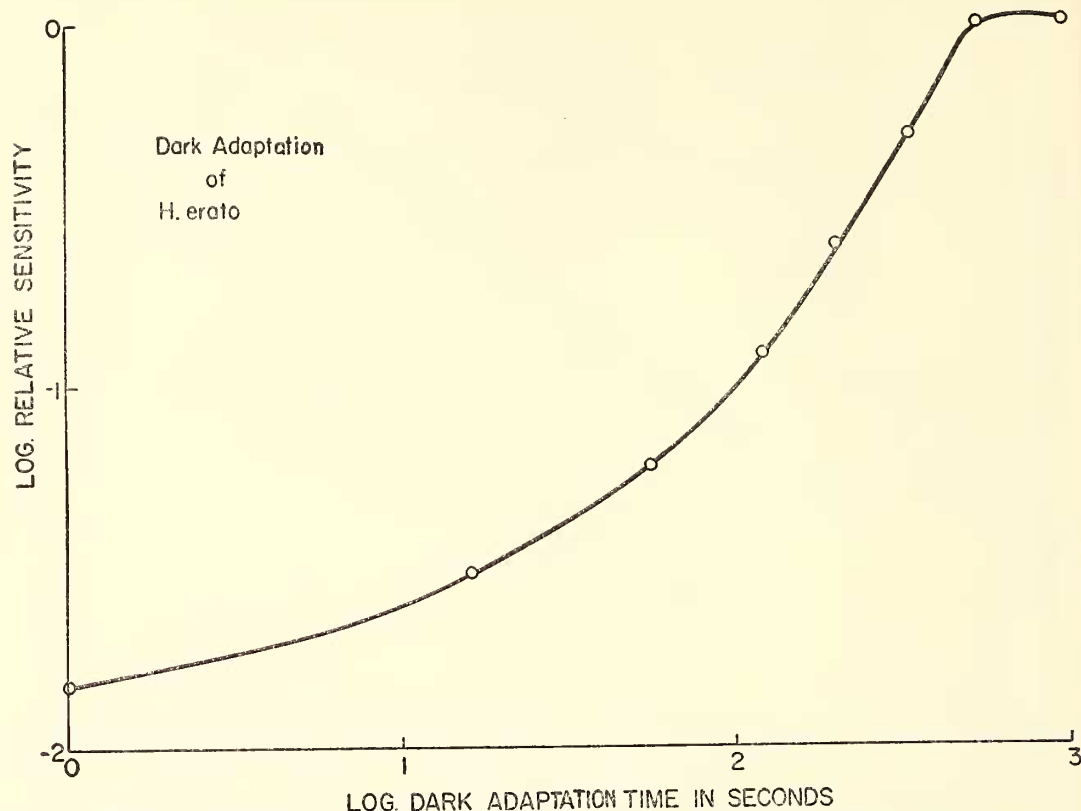
TEXT-FIG. 3. Dark adaptation curve based upon the responses of three individuals in the "day" phase. Figures were obtained by determining the minimum stimulus intensity necessary to produce a threshold electrical response, after various periods in total darkness. Eyes were light-adapted for at least 10 minutes before testing. Sensitivity did not increase after about 10 minutes' dark-adaptation, even after periods in darkness in excess of one hour.

were also observed. The activity of the butterflies was measured on the basis of the number of individuals within the sample population in flight at any given instant. The points on this curve represent the average of four such counts at fifteen-minute intervals.

Since all the females in the insectary were presumably mated, no actual mating took place during the observation period. Crane (1957) discussed, however, the irrelevant courting procedure as observed in *H. erato*. Male butterflies will frequently attempt to court mated females and will demonstrate not only the typical chase patterns, but also the usual courtship "fanning" activity until rejected by the female. The number of such courtships was taken as an index of sexual activity. The individuals within the insectary were observed continuously and each attempted courtship was recorded as belonging to one of two categories: (1) probable courtships, in which the interaction between individuals was either brief or not of a typical nature; (2) confirmed courtship attempts, which were prolonged and

demonstrated several phases of the typical courtship pattern.

The bar graph in Text-fig. 6 indicates the number of courtship attempts of both categories per half hour interval of time.

By filming some of these courtship attempts at 64 frames per second, it was possible to accurately determine the frequency of the wing beat during fanning activity. This was found to be remarkably constant and was at a frequency of 13 cycles or 26 wing strokes per second.

CONCLUSION

Based upon the foregoing, it seems clear that the ERG as recorded in the "day" phase and that recorded in the "night" reflect fundamentally different visual processes. It would appear that the "night" response is in essence a generalized photic response in which certain types of information have been sacrificed in order to attain maximum responsiveness to a minimum change in stimulus intensity. Because of the two maxima presented by the "night" luminosity curve, it
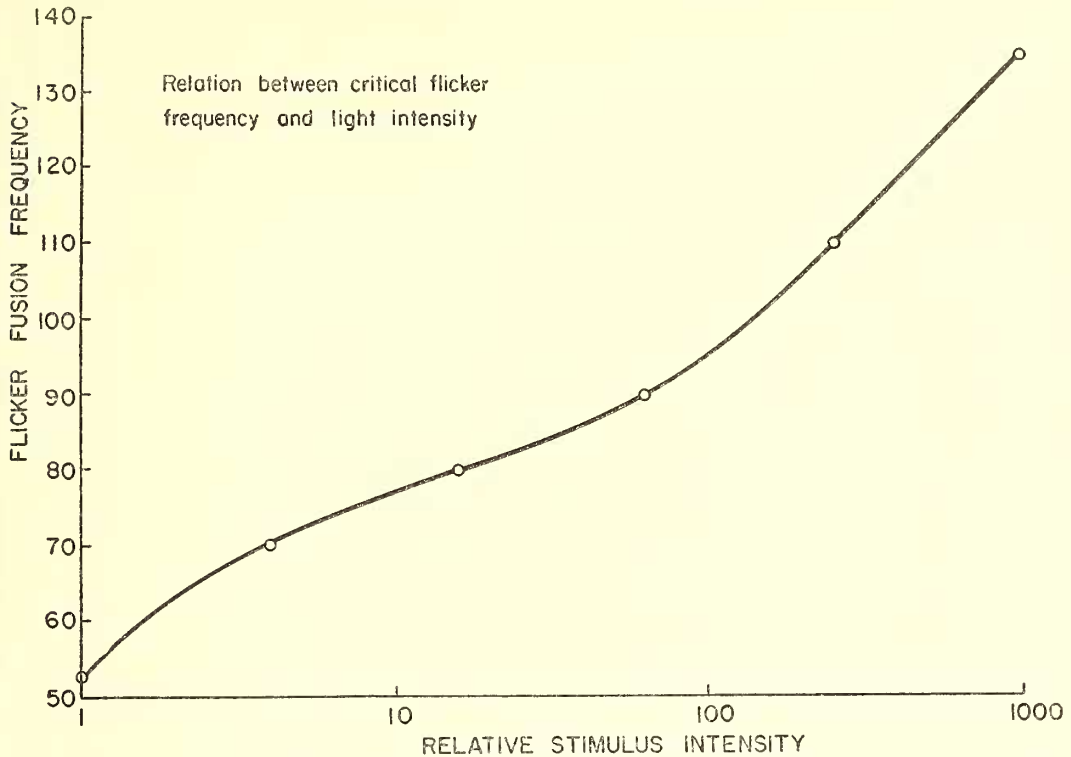
TEXT-FIG. 4. Flicker Fusion Frequency (F.F.F.) as a function of white-light stimulus intensity. Response of a fairly typical individual in "day" phase. Relative stimulus intensity of 1,000 equal to about 75,000 micro-watts in the 400 mμ to 700 mμ portion of the spectrum.

would seem logical to presume that several pigments (or receptors) are involved in producing the initial response.

The "day" response, on the other hand, appears to have a well defined ability for resolving the spectral characteristics of the stimulus. The different luminosity curves obtained from "B" and "D" waves of the "day" response would indicate that several receptors are being utilized, each producing different components of the ERG, and that the differential responses characteristic of these components are being integrated in such a manner as to produce color information at the expense of some photo-sensitivity.

The curves presented in Text-fig. 6 indicate quite clearly that the courtship behavior of *H. erato* is not related temporally to the flight activity or to such environmental conditions as light or temperature; that is to say, the maximum courtship efforts do not, in fact, come simultaneously with the maximum flight activity or with a maximum of light or temperature. We must therefore look in other directions to determine what factors control the time of courtship activity.

Attention is directed to the close similarity

between the 20-26 cycle per second frequency at which the "day" response to a flickering light becomes an "off" response, and the 26 stroke per second frequency characteristic of courtship wing flutter.

SUMMARY

The experimental work suggests an intimate and fundamental correlation between the observed electrical phenomena and basic behavior patterns. It would appear that at some critical time during the day a biological clock mechanism alters the visual mechanism in such a way that the butterfly is now prepared to receive the sensory information required in releasing the innate courtship behavior. Thus, in the morning it rather suddenly gains an "awareness" of color and an increased ability to perceive rapidly moving (fluttering) objects. With the transition to the "night" phase, its ability to respond to courtship releasers disappears. The wide variation between individuals in the time of this second transition may perhaps be related to the individual's physiological state—whether it is young or old, mated or unmated, etc. Further experiments
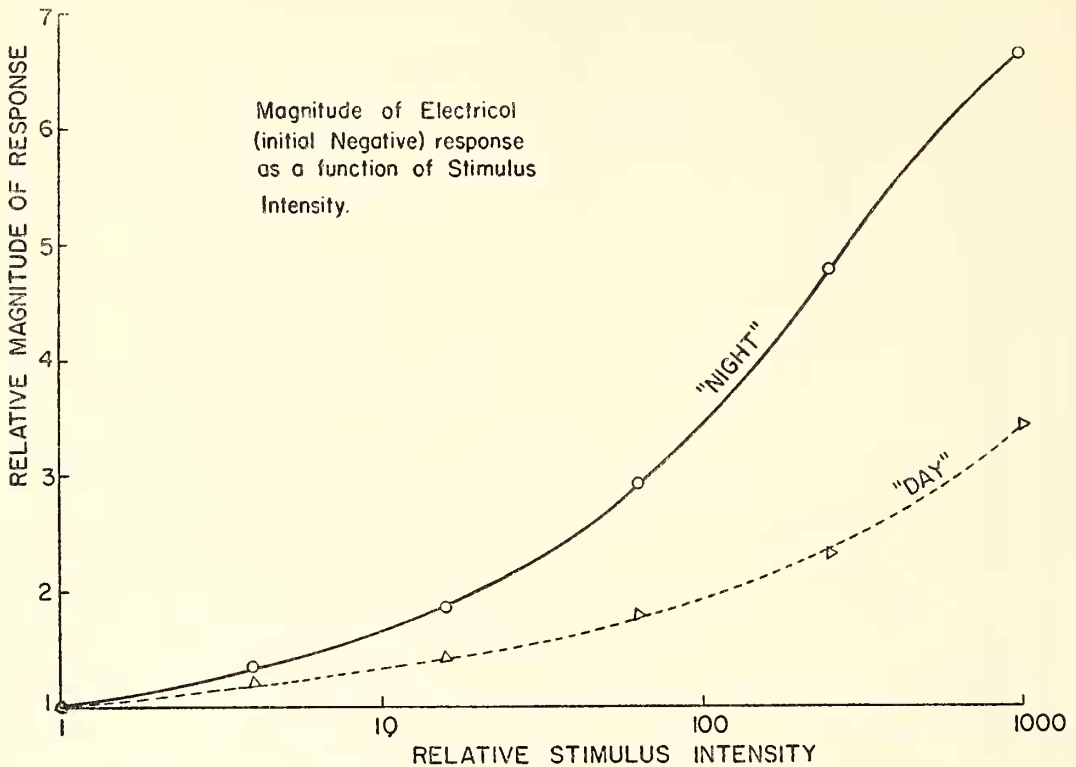
Text-fig. 5. Relationship between white-light stimulus intensity and magnitude of B wave electrical response, with the response to the weakest stimulus used, arbitrarily set at 1. Each curve represents the values obtained by averaging the responses of 11 individuals. A relative stimulus intensity of 1,000 is equal to about 75,000 micro-watts in the 400 mμ to 700 mμ portion of the spectrum.

are planned to elucidate any possible relationship of this nature.

It is indeed quite attractive to speculate that this "clock" mechanism may regulate much more than just visual processes. Alexander (1961) reports that "before 9:00 A.M." is the most common time for emergence of the adult from the pupal form. She also gives this same period of time (0800 to 1030) as the usual time of shedding the last larval skin (pupation).

Various workers (*e.g.*, Autrum & Gallwitz, 1951) have concluded that the "off" response is an inhibitory nervous component and that it suppresses the activity of the primary receptors. I agree with this point of view and will demonstrate in a forthcoming paper that the initial negative response, characteristic of the "day" eye, contains an excitatory nervous component which effectively increases the magnitude of the response of the eye to any given stimulus. With these facts in mind, it becomes quite clear that the transition from "on" to "off" responses characteristic of the response to various flicker frequencies is an extremely significant neurological phenomenon. This, in essence, constitutes

the transition between increased responsiveness and decreased responsiveness as compared with the magnitude of the response elicited by the primary receptors.

Magnus's observations concerning the responsiveness of male butterflies to certain frequencies of colored flickering light indicate that flicker rates play a role in species recognition. The correlation between the wing beat frequency and the transition to increased photic stimulation appears to be too close for mere accident. It may indeed be that the fluttering activity associated with courtship behavior serves, in part, as a visual releaser for the final phases of courtship activity.

It is expected that further research into the sensory electrophysiology of the heliconiine butterflies will provide further positive correlations between their complex behavior patterns and basic neuro-physiological mechanisms.

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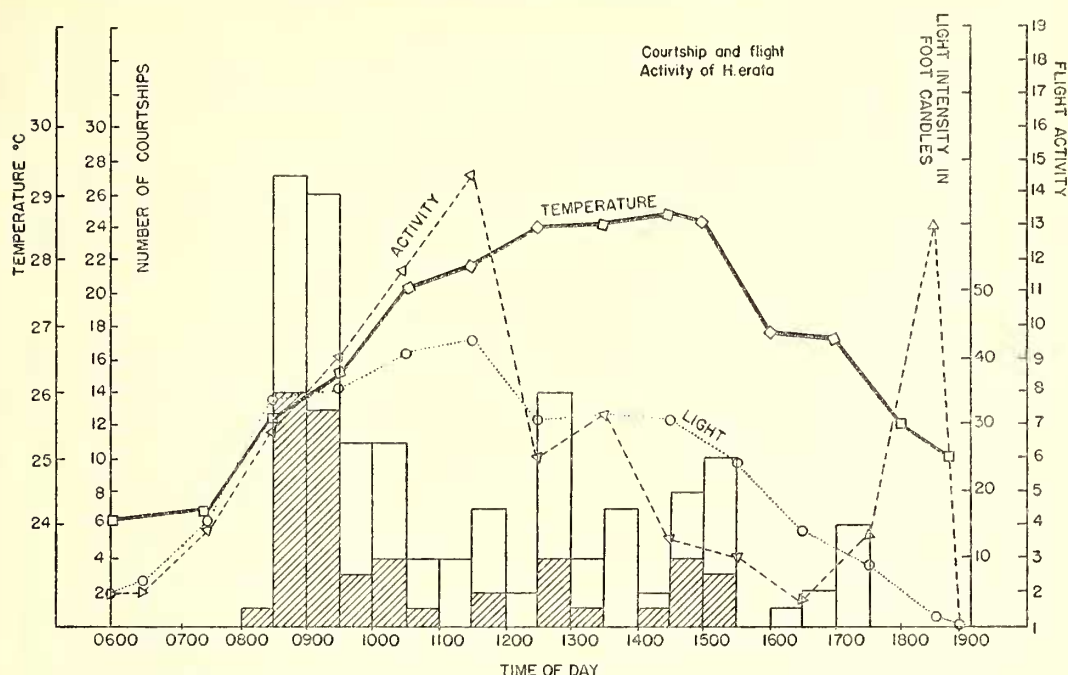
TEXT-FIG. 6. Plot of courtship behavior, flight activity, and environmental conditions, based upon observations of *ca.* 50 individuals within a large outdoor insectary, throughout one day. The bar graph represents courtship activity (see text), hatched bars depict positive courtship attempts, while unshaded bars represent questionable, or brief, attempts. Height of bars equal to total number of attempts per half hour period of time.

Light intensity is incident light in open shade as measured with a Weston model 756 illumination meter.

The flight activity is based upon the total number of individuals in flight at a given instant.

Light intensity, air temperature and flight activity were measured at 15-minute intervals, four such determinations were averaged, and hourly figures plotted.

The peak of flight activity at 1830 is due to the social roosting behavior of *H. erato.*

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EXPLANATION OF THE PLATES

Plate I

FIG. 1. Selected ERGs recorded with 15μ sub-corneal, steel electrode; D.C. amplification. Such records were made at hourly intervals throughout the day from individuals removed from the large insectaries. The 0600, 0800 and 2400 responses are typically "night" in character. The other records are "day"-type responses. Lower trace indicates period of stimulation (100 msec). A similar recording technique was employed in producing the ERGs presented in subsequent figures.

FIG. 2. Three recordings from one individual, demonstrating the changing response to white light at different times of the day (0800, 0900 and 0930). The butterfly was maintained in total darkness (except for 100 msec test flashes, at 15 minute intervals) and constant temperature, 68° F. The first recordings (0800) is clearly transitional in nature. The 0900 recording illustrates the development of the "dip," *i.e.,* return to, or below, the baseline, following the B wave. Also shown is the development of a C wave instead of a nearly constant, sustained negativity. The last ERG demonstrates the development of a pronounced "off" effect, and is a "typical" day response.

FIG. 3. ERG responses of three individuals to white-light stimulation of 100 msec. duration, after being maintained under constant environmental conditions of 220 ft-c shadowless illumination and 84° F. for various periods of time before subjecting to experimentation. The individual sampled at 0800 was so maintained for 65 hours, the 0830 for 46.5 hours, and the 1000 individual for 67 hours. In spite of deprivation of normal environmental stimuli, these butterflies demonstrate a typical diurnal pattern, with the one tested at 0800 producing a "night" response; the 0830, a transitional, and the 1000 a "day" response.

FIG. 4. Superimposed ERGs from an individual producing a day response, to three different wavelengths (616 mμ, 528 mμ and 420 mμ) with stimulus intensities adjusted to produce nearly equal magnitude B wave responses. Relative stimulus energies were 4:1:1 respectively. Stimulus duration 100 msec.

The response to red demonstrates the greatest "dip" following the B wave (falling below the lower trace) and the largest D wave or "off" effect. The blue stimulus (420 mμ), produced the smallest dip and D wave. Blue-Green (528 mμ) elicited a response intermediate in both respects.

Plate II

FIG. 5. Four selected one second portions from one continuous recording of the response of a "day" eye to flickering white light. Stimulus energy about 20,000 micro-watts, in the 400 to 700 mμ portion of the spectrum.

Flicker was produced by a rotating, sectored disk, with a gradually decreasing rotational velocity. Periods of darkness are equal to periods of stimulation. Flicker frequency was monitored by a photo-cell (lower trace). Elevated portions of the lower trace indicate periods of stimulation.

Portion A of recording: The initial response to a rapid flicker (140 cps) is identical to the day response to steady illumination. The gradual decline in the level of the base line is due to the use of a 1 cps filter.

B: Illustrates the F.F.F. or development of discrete electrical responses to each individual flash, at 110 cps.

C: Illustrates the development of an "off" response. On the left side of the figure, the beginning of the electrical response coincides with the beginning of stimulation, while toward the right, at about 25 cps, a second component begins to develop, which causes the electrical response to preceed the stimulus.

D: Illustrates how this second component gradually increases in importance until the response becomes entirely "off" in nature.

FIG. 6. Superimposed ERGs from an individual producing a night response, to the same three wavelengths used for Fig. 4 (616 mμ, 528 mμ and 420 mμ). Stimulus duration 100 msec. Intensities adjusted to produce nearly equal magnitude initial negative responses. Relative stimulus intensities 2:1:2, respectively. Waveforms elicited are essentially identical.